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## ORIGINAL ARTICLE

# Nutrient and mineral composition during shoot growth in seven species of *Phyllostachys* and *Pseudosasa* bamboo consumed by giant panda

A. L. Christian<sup>1</sup>, K. K. Knott<sup>1</sup>, C. K. Vance<sup>1,2</sup>, J. F. Falcone<sup>1</sup>, L. L. Bauer<sup>3</sup>, G. C. Fahey Jr<sup>3</sup>, S. Willard<sup>2</sup> and A. J. Kouba<sup>1</sup>

<sup>1</sup> Conservation and Research Department, Memphis Zoological Society, Memphis, TN, USA

<sup>2</sup> Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University MS, USA, and

<sup>3</sup> Animal Sciences Department, University of Illinois, Urbana-Champaign, IL, USA

## Summary

During the annual period of bamboo shoot growth in spring, free-ranging giant pandas feed almost exclusively on the shoots while ignoring the leaves and full-height culm. Little is known about the nutritional changes that occur during bamboo shoot growth, if nutritional changes differ among species, or how these changes might influence forage selection. Our objective was to examine the nutrient and mineral composition during three phases of shoot growth (<60, 90–150 and >180 cm) for seven species of bamboo (*Phyllostachys (P.) aurea*, *P. aureo-sulcata*, *P. bissetii*, *P. glauca*, *P. nuda*, *P. rubromarginata*, *Pseudosasa japonica*) fed to captive giant pandas at the Memphis Zoo. Total dietary fiber content of bamboo shoots increased ( $p < 0.0001$ ) from an overall species average of 61% dry matter (DM) at < 60 cm to 75% DM at shoot heights > 180 cm, while crude protein, fat and ash exhibited significant declines ( $p < 0.05$ ). *Phyllostachys nuda* had the overall greatest ( $p = 0.007$ ) crude protein (21% DM) and fat (4% DM) content, and lowest overall total fibre (61% DM) content compared to the other species examined. In contrast, *Pseudosasa japonica* had the overall lowest crude protein and fat, and relatively higher fibre content (9%, 3% and 74% respectively). Concentrations of Zn and Fe were highest in shoots <60 cm (10–50  $\mu\text{g/g}$  DM) and decreased ( $p < 0.05$ ) during growth in all species examined. Concentrations of Ca, Cu, Mn, Na and K varied among species and were largely unaffected by growth stage. Due to their higher concentrations of nutrients and lower fibre content in comparison to culm and leaf, bamboo shoots should be a major component of captive giant panda diets when available.

**Keywords** fibre, lignin, nutrient value, selective foraging, trace minerals

**Correspondence** K. K. Knott, Memphis Zoo, 2000 Prentiss Place, Memphis, TN 38112, USA. Tel: 901-333-6721; Fax: 901-333-6671; E-mail: kknott@memphiszoo.org

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## Introduction

Bamboo is an exclusive dietary option for many animals that inhabit bamboo forests due to its rapid growth, annual regeneration and tendency to reduce the development of surrounding vegetation. Specialized bamboo foragers, such as the giant panda (*Ailuropoda melanoleuca*), red panda (*Ailurus fulgens*), bamboo lemur (genus *Hapalemur*) and bamboo rats (genus *Rhizomys* and *Cannomys*), have various adaptations to survive almost entirely on this high-fibre forage (Schaller et al., 1985). Of particular interest is the endangered giant panda, which exists in the wild as an obligate bamboo forager although it has the gastrointestinal tract of a carnivore. Due to its short gut and

rapid passage rate of digesta, the giant panda has been estimated to digest only 17% of bamboo dry matter and cannot efficiently utilize all of the available nutrients (Dierenfeld et al., 1982). To meet its daily dietary requirements, the giant panda must consume excessive amounts of bamboo (6–15% of its body weight daily) and reduce its energy expenditure by resting for extended periods throughout the day (Dierenfeld, 1997). Although equipped with a gastrointestinal tract that is inefficient at digesting fibre, several reports have identified gut microflora unique to giant pandas that may aid in fibre fermentation to an extent (Hirayama et al., 1989; Zhu et al., 2011; Williams et al., 2013). Selective feeding behaviours may also help giant pandas meet their nutritional requirements

(Schaller et al., 1985; Long et al., 2004; Hansen et al., 2010). For example, giant pandas selectively forage on different parts of bamboo and select different bamboo species depending on the season, elevation and the individual's physiological state (e.g. growth and development, reproductive status, lactation). Giant pandas prefer early shoots over full-height culm and leaf during the bamboo-shooting season in spring. As such, bamboo shoots have been hypothesized to provide a vital nutritive opportunity to improve body condition and reproductive fitness (Schaller et al., 1985; Long et al., 2004).

Bamboo is an extremely fibrous plant, consisting mostly of the highly stable polymers cellulose, hemicellulose and lignin that provide its characteristic structural support. Complete lignification of shoots typically occurs during the first year of growth. The high-fibre content of full-height culm and leaves may reduce the bioavailability of proteins, fats, minerals and sugars for bamboo consumers that lack specialized compartments for fibre fermentation (Van Soest, 1975, 1977; Schaller et al., 1985). Bamboo shoots are considerably less fibrous than full-height culms primarily because the plant cell walls have not yet fully developed. Therefore, shoots are more easily digested, and nutrients may be more bioavailable (Wei et al., 2000; Halvorson et al., 2010). Although changes in nutrient content of full-height culm and leaves have been well reviewed, few studies have examined the nutritional components of bamboo shoots during early growth phases. Nutritional information on shoot growth has primarily focused on shoots of *Bambusa* and *Dendrocalamus* spp. grown for human food consumption or as commercial products in India, and shoots were only examined at extremely early growth phases (<10 cm, 2–20 days after emergence; Nirmala et al., 2007; Chang et al., 2013; Pandey and Ojha, 2013). Although Halvorson et al. (2010) described some general changes in nutritive value of *Phyllostachys* shoots in relation to leaves that study did not completely describe variations among species. There remains a scarcity of data available regarding the changes in nutrient content during early shoot growth and culm elongation in those bamboo species that are commonly provided to giant pandas in captivity.

Bamboos provided to giant pandas in captivity include the cold-tolerant species under the genera *Phyllostachys* and *Pseudosasa* that are successfully grown in the United States. *Phyllostachys* species belong to a group of bamboo characterized by horizontal leptomorph rhizomes that spread rapidly underground, earning them the moniker 'running'

bamboos. *Phyllostachys* species are generally less fibrous than their 'clumping' bamboos counterparts with pachymorph rhizomes such as the tropical *Bambusa* group (Liese, 1992; Makita, 1998; Halvorson et al., 2010). In general, *Phyllostachys* (*P.*) bamboos that are commercially cultivated are advertised to grow >9 m, and *P. aurea*, a species analysed in this study, has been reported to reach a full height of approximately 7 m in 1 month (Suzuki and Itoh, 2001). *Pseudosasa* (*Ps.*) *japonica* is another example of a running bamboo species that thrives in temperate climates, although it does not grow to the same heights as the *Phyllostachys* bamboos reaching only approximately 2–3 m (Triplett and Clark, 2010). Preference for *Ps. japonica* has been observed in giant pandas at the Smithsonian National Zoological Park and Memphis Zoo in the USA (Tarou et al., 2005; A. J. Kouba, unpublished data). The accepted feeding practice by U.S. zoos maintaining giant pandas is to offer full-height culms and leaves from a variety of bamboo species *ad libitum*, year round (Dierenfeld et al., 1995; Hansen et al., 2010; Williams et al., 2013). Although it has been recorded that wild giant pandas will consume a diet comprised almost completely of bamboo shoots during shooting season (Schaller et al., 1985; Dierenfeld, 1997), there are no reports describing how that phenomena have been replicated for animals in captivity and the potential nutrient value of such feeding practices. In 2013, keeper records of the Memphis Zoo indicated that the offered diet consisted of approximately 18% shoots for the female giant panda and 10% for the male during the months of April and May (B. Gocinski, unpublished data), which exceeded the amount of shoots offered for all other zoos holding pandas in North America. While bamboo shoots appear to be an integral facet of the wild giant panda diet, their role in giant panda nutritional health and reproduction has not been well reviewed.

The objectives of this study were to examine the relationship between bamboo shoot growth and nutritional value and to describe the differences in nutrient composition among seven species of bamboo shoots (*P. aurea*, *P. aureosulcata*, *P. bissetii*, *P. glauca*, *P. nuda*, *P. rubromarginata* and *Ps. japonica*) consumed by giant pandas at the Memphis Zoo. It was hypothesized that as shoots elongate over the growing season that the nutrient value will decrease. This will be evident in a decrease of crude protein, fat, and mineral content and an increase in fibre content. Nutritional data from bamboo shoots are also presented in comparison with previous studies describing the nutrient value of full-height culm and leaves. Information resulting from this study will apply to the nutrition and husbandry

protocols of obligate herbivores with diets consisting primarily of bamboo, particularly the giant panda.

## Materials and methods

### Sample collection and preparation

Shoots from seven species of bamboo (*P. aurea*, *P. aureosulcata*, *P. bissetii*, *P. glauca*, *P. nuda*, *P. rubromarginata* and *Ps. japonica*) were harvested from the Memphis Zoo bamboo farm (Agricenter, Memphis, TN) during April–May over a period of 52 days during 2009 and 38 days in 2010. Shoot heights representing early culm emergence and elongation were grouped into the following categories: <60, 90–150 and >180 cm. Shoots developing branches or leaves were not considered. Multiple shoots (7–9 kg wet mass) were taken from random locations within each species plot for each growth stage. Shoots were cut into approximately 1.2–2.5 cm segments and dried at 60 °C for 48 h in a forced air oven. The entire bundle of dried shoots for each species and height category was ground through a Wiley Mill Model 4 (Thomas Scientific, Swedesboro, NJ, USA) to pass a 1 mm screen, and a subsample of the combined mixture was used for nutritive analyses.

### Chemical analyses

Dried ground samples (50–100 g) were analysed at the University of Illinois at Urbana-Champaign (Urbana, IL) for macronutrients (2009 and 2010 samples), individual fibre components (2009 samples only) and minerals (2009 samples only). A ground subsample was weighed and dried at 100 °C for 24 h to determine dry matter (DM) content of the dried ground sample (AOAC, 1995). The following nutrient concentrations were calculated in reference to total DM. Total ash was determined by combustion of a subsample in a furnace at 500 °C for 12 h. Organic matter (OM) was calculated by subtraction of ash from DM. Crude protein (CP = % total nitrogen  $\times$  6.25) was assessed by a Leco Nitrogen Analyzer (model FP-2000, Leco Corporation, St. Joseph, MI) (AOAC, 1995). Acid-hydrolysed fat (AHF) was analysed by the standard procedure of acid hydrolysis with HCl, followed by ether extraction as described previously (Budde, 1952). Total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) were measured using the enzymatic-gravimetric methods defined by Prosky *et al.* (1985, 1992). Total dietary fibre was determined by enzymatic digestion of a subsample using Termamyl<sup>®</sup> (a heat stable  $\alpha$ -amylase), proteases and amyloglucosidase, followed

by filtration with washes of ethanol and acetone. Once the residue was dried, ash and CP concentrations were determined as described above and those values subtracted to yield TDF. To measure IDF, another subsample was enzymatically digested (Termamyl<sup>®</sup>, protease and amyloglucosidase) and filtered with washes of H<sub>2</sub>O (to remove soluble fibre), ethanol and acetone. Residual ash and CP were determined and subtracted to yield IDF. Soluble dietary fibre was calculated as the difference between TDF and IDF. The acid detergent fibre (ADF) method determined the combined cellulose and lignin content of the sample by extraction in an acid detergent solution. Lignin content was estimated by Van Soest's acid detergent lignin (ADL) method in which H<sub>2</sub>SO<sub>4</sub> hydrolyses the cellulose component of the ADF, isolating any remaining lignin (Van Soest, 1963). A dried and ashed subsample was extracted in 20% HCl for determination of calcium (Ca), potassium (K), sodium (Na), zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) by atomic absorption spectroscopy (Nelson *et al.*, 2000). Data for macromineral content were reported as mg/g DM, and trace minerals were reported as  $\mu$ g/g DM.

### Statistical analyses

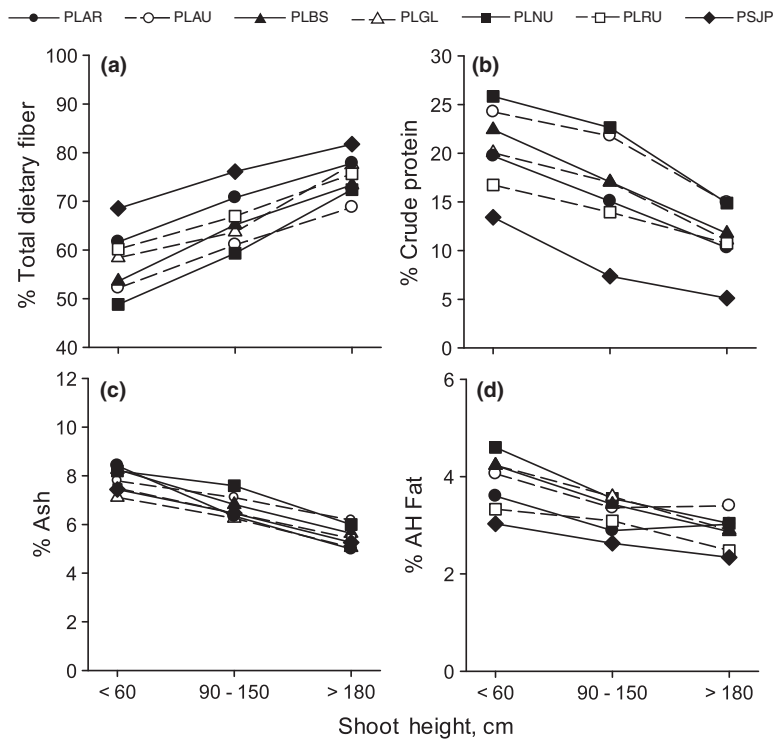
All samples were selected randomly across each species stand and considered to be independent samples. A mixed model analysis of variance (ANOVA) was used to determine whether each analyte differed with respect to the fixed effects of species and growth phase, and the random effect of growth year (2009 or 2010). Data sets were examined for normal distributions using the Shapiro–Wilks test. In the case of ADL where the raw data did not fit a normal distribution, an arcsine transformation was used (Quinn and Keough, 2002). No transformation improved the distribution for K and Mn; therefore, the differences in species and growth stage for these minerals were determined using the non-parametric Kruskal–Wallis test. A Tukey's HSD (honest significant difference) test was used for pairwise multiple comparisons and differences were considered significant at  $p < 0.05$ . Statistical analyses were performed using the program JMP 9.0.2 (SAS Institute Inc., Cary, NC) and graphs prepared with SigmaPlot 10.01.25 (Systat Software, Inc., San Jose, CA).

## Results

### Macronutrients

#### Year

Bamboo collected during 2010 had lower ash content than bamboo shoots examined during 2009 (Figs 1



**Fig. 1** Percentage of (a) total dietary fibre, (b) crude protein, (c) ash and (d) acid-hydrolysed (AH) fat during three stages of shoot growth for seven species of bamboo: *Phyllostachys aurea* (PLAR), *Phyllostachys aureosulcata* (PLAU), *Phyllostachys bissetii* (PLBS), *Phyllostachys glauca* (PLGL), *Phyllostachys nuda* (PLNU), *Phyllostachys rubromarginata* (PLRU), *Pseudosasa japonica* (PSJP) collected in 2009. There was a significant difference in nutritional content by shoot size ( $p < 0.0001$ ) and by species ( $p < 0.05$ ) for all nutrients measured.

and 2); thus, subsequent analyses of these macronutrients were performed separately for 2009 and 2010. Growth year was not significant for TDF, CP or AHF ( $p = 0.43$ ,  $p = 0.16$ ,  $p = 0.74$  respectively); thus, the ANOVA was performed on the combined data set as described below.

#### Growth phase

Shoot growth, as represented by shoot height, was a significant source of variance ( $p < 0.05$ ) for all macronutrient data. As was hypothesized, all species exhibited an increase of TDF (Figs 1–3) with shoot elongation. In all species, ADF and ADL content were also greater in shoots exceeding 180 cm than shoots at younger growth stages ( $p < 0.05$ ). The TDF, which ranged between 45 and 80% of DM, was composed of 95–100% insoluble dietary fibre (IDF) and <5% soluble dietary fibre (SDF) for all species and growth stages. Conversely, CP, ash and AHF decreased as shoots grew taller (Figs 1–3;  $p < 0.0001$ ).

#### Species

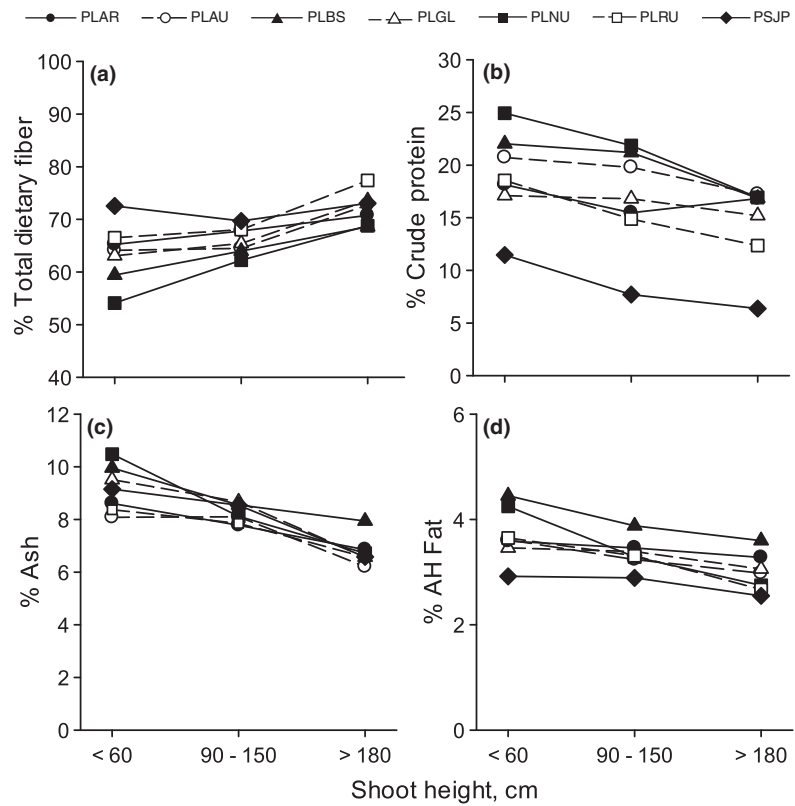
Overall, *Ps. japonica* had lower CP (8.6% vs. 17.7%) and AHF (2.7% vs. 3.4%) and greater TDF (73.6% vs. 65.7%) than the combined average for *Phyllostachys* species (Figs 1 and 2; Table 1). Acid detergent fibre (ADF) and lignin (ADL) content of *Ps. japonica* were also greater ( $p < 0.0001$ ) at all stages of growth than

the *Phyllostachys* species (combined averages; ADF = 42.4% vs. 32.5%; ADL = 5.9% vs. 2.5%; Table 1). In contrast, the combined growth stage average of *P. nuda* shoots had the greatest CP (21.2%) and AHF (3.6%) and only 61.0% TDF while the combined average for all other bamboo species was 15.6% CP, 3.3% AHF and 67.8% TDF (2009 and 2010; Figs 1–3; Table 1). The percentage of lignin in *P. nuda* shoots was also lower ( $p < 0.001$ ) at all stages of growth than the other species (Table 1).

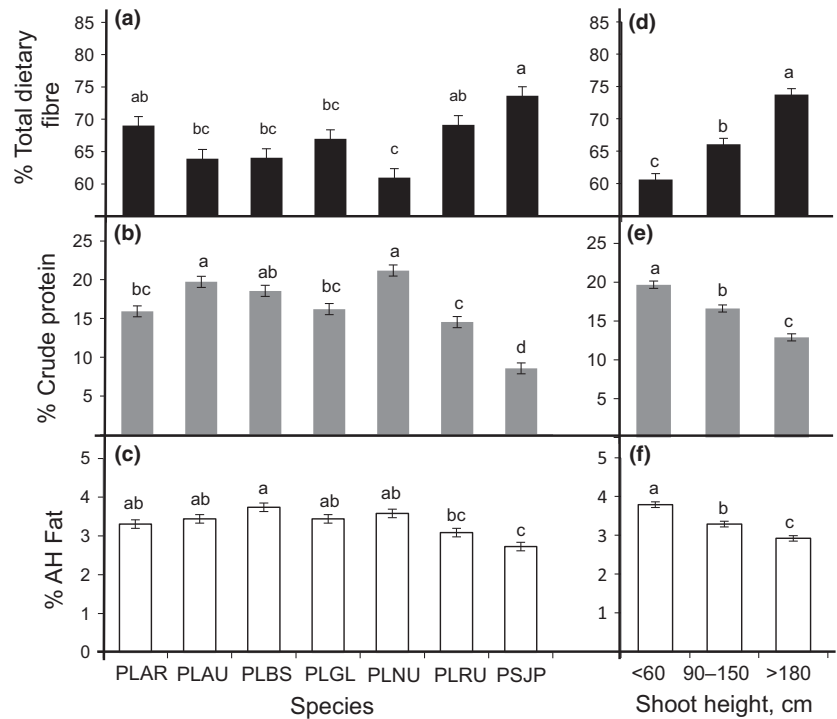
#### Minerals

##### Macrominerals

The macrominerals K, Ca and Na did not differ appreciably with growth or species ( $p > 0.31$ ; Fig. 4; Table 2). Nonetheless, some interesting patterns were observed in the data. For example, *P. nuda* was two times greater in K at shoot heights <60 and 90–150 cm (72.7–96.3 mg/g) than the other species, although K at >180 cm decreased to levels approximating other species (Fig. 4a). All other bamboo species displayed similar initial levels of K concentration (35.5–43.2 mg/g) and shared a broader range of concentrations at heights of 90–150 and >180 cm (28.5–62.0 mg/g; Fig. 4a; Table 2). Concentrations of Ca for *P. rubromarginata* was nearly twice the amount of Ca compared to other species in the smallest and



**Fig. 2** Percentage of (a) total dietary fibre, (b) crude protein, (c) ash and (d) acid-hydrolysed (AH) fat (AHF) during three stages of shoot growth for seven species of bamboo: *Phyllostachys aurea* (PLAR), *Phyllostachys aureosulcata* (PLAU), *Phyllostachys bisetii* (PLBS), *Phyllostachys glauca* (PLGL), *Phyllostachys nuda* (PLNU), *Phyllostachys rubromarginata* (PLRU), *Pseudosasa japonica* (PSJP) collected in 2010. There was a significant difference in nutritional content by shoot size ( $p < 0.0001$ ) and by species ( $p < 0.05$ ) for all nutrients measured.



**Fig. 3** Percentages of (a) total dietary fibre (TDF), (b) crude protein (CP) and (c) acid-hydrolysed (AH) fat (mean  $\pm$  SEM) for each species (*Phyllostachys aurea* (PLAR), *Phyllostachys aureosulcata* (PLAU), *Phyllostachys bisetii* (PLBS), *Phyllostachys glauca* (PLGL), *Phyllostachys nuda* (PLNU), *Phyllostachys rubromarginata* (PLRU), *Pseudosasa japonica* (PSJP)). Percentages of TDF (d), CP (e) and AHF (f) for all species combined in relation to stage of shoot growth (<60, 90-150, >180 cm). Data represent pooled sample means from 2009 and 2010. Lower case letters indicate similarity within a panel.

**Table 1** Comparison of fibre (%; TDF, total dietary fibre; ADF, acid detergent fibre; ADL acid detergent lignin), crude protein (%CP), ash (%) and fat (% AHF) content in shoots, leaves and culm of *Phyllostachys* (*P.*) and *Pseudosasa* (*Ps.*) bamboo. All values are expressed as % of DM. Values from previously published studies are shown as the average, whereas data from the present study are shown as the range to reflect the variation of the three growth stages analysed

Species	Part	TDF	ADF	ADL	CP	Ash	AHF	Source
<i>P. aurea</i>	Shoot	61.6–77.8	31.2–43.1	2.5–3.8	10.3–19.7	5.0–8.4	2.9–3.1	Present study
<i>P. aurea</i>	Shoot	–	32.1	2.7	21.3	–	–	Mainka et al. (1989)
<i>P. aureasulcata</i>	Shoot	52.2–68.8	21.0–36.1	1.3–3.1	14.9–24.3	6.2–7.8	3.4–4.1	Present study
<i>P. aureasulcata</i>	Shoot	–	22.5	1.2	23.4	0.3	–	Tabet et al. (2004)
<i>P. bissettii</i>	Shoot	53.6–73.4	24.4–41.7	2.1–3.5	11.8–22.4	5.6–8.3	2.9–4.2	Present study
<i>P. bissettii</i>	Shoot	–	26.6	1.8	18.6	0.8	–	Tabet et al. (2004)
<i>P. glauca</i>	Shoot	58.4–77.6	35.3–43.4	2.5–4.3	11.1–20.1	5.1–7.1	2.9–4.2	Present study
<i>P. nuda</i>	Shoot	48.8–72.4	21.9–39.1	1.0–2.2	14.9–25.8	6.0–8.2	3.0–4.6	Present study
<i>P. rubromarginata</i>	Shoot	60.2–75.7	29.4–41.2	2.3–3.0	10.8–17.7	5.4–7.5	2.5–3.3	Present study
<i>Ps. japonica</i>	Shoot	68.6–81.7	33.4–50.3	4.4–7.4	5.1–13.4	5.3–7.4	2.3–3.0	Present study
<i>Ps. japonica</i>	Shoot	–	34.6	3.8	11.5	1.9	–	Tabet et al. (2004)
<i>P. aurea</i>	Leaf	–	32.8	2.7	19.8	–	–	Mainka et al. (1989)
<i>P. aurea</i>	Culm	–	57.1	3.8	5.0	–	–	Mainka et al. (1989)
<i>P. aureasulcata</i>	Leaf	–	27.7	6.2	13.4	13.4	–	Dierenfeld et al. (1982)
<i>P. aureasulcata</i>	Leaf	–	34.8	10.4	15.5	7.4	–	Halvorson et al. (2010)
<i>P. aureasulcata</i>	Culm	–	44.2	9.9	3.8	2.3	–	Dierenfeld et al. (1982)
<i>P. aureasulcata</i>	Culm	–	48.2	7.1	2.2	1.2	–	Dierenfeld et al. (1982)
<i>P. bissettii</i>	Leaf	–	34.8	5.8	12.9	7.1	–	Tabet et al. (2004)
<i>P. bissettii</i>	Leaf	–	34.2	10.5	16.5	8.7	–	Halvorson et al. (2010)
<i>P. nuda</i>	Leaf	–	34.7	9.5	15.3	6.9	–	Halvorson et al. (2010)
<i>P. rubromarginata</i>	Leaf	–	36.2	10.8	15.6	7.8	–	Halvorson et al. (2010)
<i>Ps. japonica</i>	Leaf	–	41.3	6.2	11.5	7.2	–	Tabet et al. (2004)

mid-sized shoots, but as the shoots elongated Ca concentrations were similar for all species (Fig. 4b). Younger growth stages of *P. glauca*, *P. aurea* and *P. rubromarginata* shoots had nearly double the Na concentrations (0.4–0.5 mg/g) compared with other species of the same size (Fig. 4c).

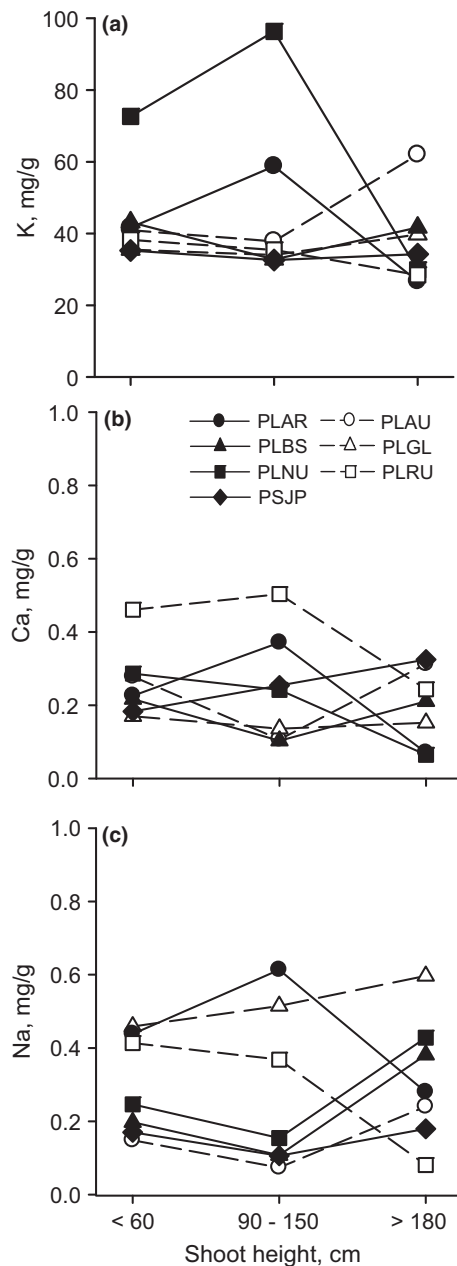
#### Trace minerals

Concentrations of Mn differed ( $p < 0.05$ ) among species with highest concentrations in *P. rubromarginata* (120–180  $\mu\text{g/g}$ ), *P. aureasulcata* (60–100  $\mu\text{g/g}$ ) and *Ps. japonica* (30–50  $\mu\text{g/g}$ ; Fig. 5a). For these three species, Mn concentrations also decreased as the shoots grew taller. Manganese concentrations for the other four *Phyllostachys* species were generally  $<30 \mu\text{g/g}$  for all growth stages with little variation between shoot growth categories. Bamboo shoot concentrations for Zn and Fe ranged from 10 to 50  $\mu\text{g/g}$  and were  $<12 \mu\text{g/g}$  for Cu (Fig. 5). Zinc and Fe decreased in all species as shoot height increased ( $p < 0.05$ ; Fig. 5). Zinc and Fe also varied by species ( $p < 0.003$ ) with the greatest Zn concentrations in *P. nuda*, *P. bissettii* and *P. rubromarginata* whereas Fe concentrations decreased by species in the following order: *P. aurea*  $\geq$  *P. nuda* = *P. glauca* = *P. aureasulcata* = *P. rubromarginata* = *Ps. japonica*  $\geq$  *P. bissettii* (Fig. 5; Table 2).

Concentrations of Cu did not differ between species (range 5.2–7.8  $\mu\text{g/g}$ ; Fig. 5d; Table 2). Although a decrease in Cu with increasing shoot height approached significance ( $p = 0.07$ ), this trend was likely due to the high Cu values observed for *Ps. japonica* at  $<60 \text{ cm}$  and low Cu values for *P. rubromarginata* at  $>180 \text{ cm}$  (Fig. 5d).

#### Discussion

In grasses grown for hay or pasture, indigestible plant matter increases with plant growth and maturity (Van Soest and Wine, 1967; Darlington and Hershberger, 1968; Cherney et al., 1993; Hoffman et al., 1993; Nelson and Moser, 1994). Similar plant compositional changes associated with growth of bamboo shoots were observed in the present study. Based on crude protein, fat, ash and fibre, shoots of *Phyllostachys* and *Pseudosasa* bamboo exhibited the greatest nutritional value in the youngest growth phase ( $<60 \text{ cm}$ ), and values diminished as shoots elongated during spring. These results confirmed earlier reports that described an increase in the fibre content during early shoot emergence for *Bambusa* and *Dendrocalamus* bamboo (Nirmala et al., 2007; Chang et al., 2013; Pandey and Ojha, 2013) and that fibre content of shoots was lower



**Fig. 4** Relationship between concentrations of macro minerals (a) K, (b) Ca and (c) Na and shoot size for seven species of bamboo: *Phyllostachys aurea* (PLAR), *Phyllostachys aureosulcata* (PLAU), *Phyllostachys bissetii* (PLBS), *Phyllostachys glauca* (PLGL), *Phyllostachys nuda* (PLNU), *Phyllostachys rubromarginata* (PLRU), *Pseudosasa japonica* (PSJP) collected in 2009. Concentrations of macrominerals did not vary by shoot height, and only Na concentrations varied among species ( $p = 0.039$ ).

than leaves for *Phyllostachys* species (Halvorson et al., 2010). Pandey and Ojha (2013) and Nirmala et al. (2007) also described that decreasing concentrations of crude protein, fat and ash were correlated with increasing shoot height in *Bambusa* and *Dendrocalamus*

bamboo. Fibre and crude protein content of *Phyllostachys* and *Pseudosasa* bamboo in this study were within the range for these same species previously reported by Mainka et al. (1989) and Tabet et al. (2004) (Table 1). Moreover, this study confirmed the assumption that shoots are a more nutrient rich plant part than full-height culm and are of similar or superior nutrient value in comparison with bamboo leaves (Tables 1 and 2). It should be noted, however, that the fat content of bamboo in this study and other bamboo nutrient composition studies may be overestimated as values were determined by acid hydrolysis and ether extraction that does not exclude the waxes on the plant surface.

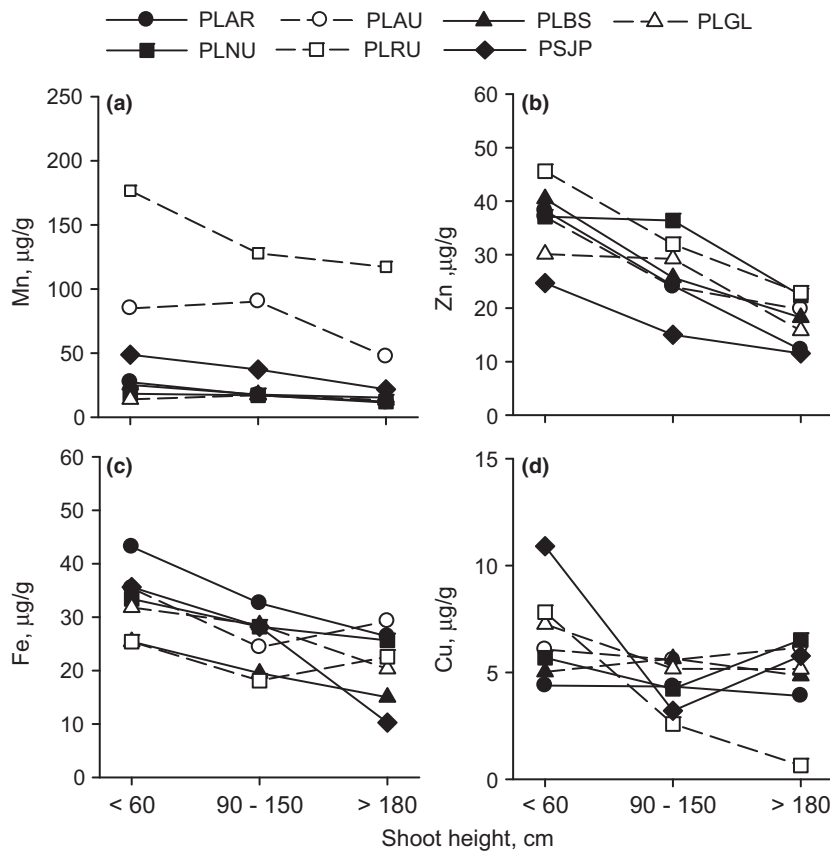
*Pseudosasa* bamboo shoots had higher fibre and lignin content, and lower crude protein, fat and ash relative to *Phyllostachys* shoots. A physical difference observed between the genera is that full-height culms of *Ps. japonica* are shorter, with a maximum culm height of 2–3 m relative to the various *Phyllostachys* bamboo that can reach culm heights >4 m. In this study, shoots of all seven species were measured and harvested at <60, 90–150 and >180 cm. Therefore, *Ps. japonica* was likely at a later growth stage at each measure of shoot height than the other species examined as *Ps. japonica* was relatively nearer to its final seasonal height. As such, it is not surprising that *Ps. japonica* would have comparably greater quantities of fibre and lower crude protein, fat and ash. Rainfall may also have influenced growth rate and consequent nutrient composition of bamboo during the two sampling years examined. Specifically, Memphis, TN received considerably less rainfall in 2009 (April, 9.2 cm; May, 19.6 cm) than in 2010 (April, 17.8 cm; May, 23.5 cm). Greater rainfall may have resulted in a lower osmotic potential to exist in the plant and consequently lower ash content in the bamboo shoots collected during 2010 versus 2009. In future studies, a more accurate species comparison of nutrient composition could be determined through a standardization of shoot height from time of emergence or as proportional to full-height culm.

Total ash, a representation of total mineral content, decreased with shoot height in both 2009 and 2010 consistent with the postulation that mineral concentrations decrease as bamboo shoots elongate during the growing season. Overall, concentrations of trace minerals were highest in very young shoots <60 cm in height and declined during shoot elongation. Decreases in Zn and Fe during shoot growth were also reported in *Bambusa* and *Dendrocalamus* bamboo (Nirmala et al., 2007), and other grass forage studies (Spears, 1994). Minerals, particularly Ca, Cu,



**Table 2** Comparison of the macromineral (mg/g; K, potassium; Ca, calcium; Na, sodium) and trace mineral ( $\mu\text{g/g}$ ; Mn, manganese; Zn, zinc; Fe, iron; Cu, copper) content in shoots, leaves and culm of *Phyllostachys* (*P.*) and *Pseudosasa* (*Ps.*) bamboo. All values are expressed on a DM basis. Values from previously published studies are shown as the average, whereas data from the present study are shown as the range to reflect the variation of the three growth stages analysed

Species	Part	K	Ca	Na	Mn	Zn	Fe	Cu	Source
<i>P. aurea</i>	Shoot	26.7–41.7	0.1–0.4	0.3–0.4	11.5–27.3	12.2–38.2	26.4–43.2	5.2–7.3	Present study
<i>P. aurea</i>	Shoot	23.3	2.7	–	–	45.0	–	35.0	Mainka et al. (1989)
<i>P. aureasulcata</i>	Shoot	37.8–62.0	0.1–0.3	0.2–0.7	47.4–85.0	19.8–37.1	24.3–35.4	3.9–4.4	Present study
<i>P. aureasulcata</i>	Shoot	36.2	0.7	–	74.7	–	–	–	Tabet et al. (2004)
<i>P. bissettii</i>	Shoot	32.8–43.2	0.1–0.2	0.1–0.4	15.4–25.3	18.3–40.5	15.0–25.4	4.2–6.5	Present study
<i>P. bissettii</i>	Shoot	34.0	1.3	–	19.0	–	–	–	Tabet et al. (2004)
<i>P. glauca</i>	Shoot	34.0–39.7	0.1–0.2	0.5–0.6	13.5–17.8	15.8–30.1	20.3–31.8	4.8–5.6	Present study
<i>P. nuda</i>	Shoot	30.0–96.3	0.1–0.3	0.2–0.4	12.3–18.4	22.4–37.1	25.6–33.4	5.6–6.2	Present study
<i>P. rubromarginata</i>	Shoot	28.5–38.3	0.2–0.5	0.1–0.4	117.4–176.7	22.9–54.6	18.1–25.4	0.6–7.8	Present study
<i>Ps. japonica</i>	Shoot	32.6–35.3	0.2–0.3	0.1–0.2	24.9–48.8	11.5–24.7	10.3–35.6	3.2–5.8	Present study
<i>Ps. japonica</i>	Shoot	26.6	1.0	–	77.1	–	–	13.0	Tabet et al. (2004)
<i>P. aurea</i>	Leaf	10.0	7.3	–	–	24.0	–	15.0	Mainka et al. (1989)
<i>P. aurea</i>	Culm	11.0	1.4	–	–	24.0	–	–	Mainka et al. (1989)
<i>P. aureasulcata</i>	Leaf	8.9	4.9	–	153.5	–	–	27.3	Tabet et al. (2004)
<i>P. aureasulcata</i>	Leaf	19.9	3.1	<0.1	27.4	14.0	332.0	–	Hunter et al. (2003)
<i>P. bissettii</i>	Leaf	9.2	7.4	–	83.8	–	–	–	Tabet et al. (2004)
<i>Ps. japonica</i>	Leaf	7.8	5.3	–	574.9	–	–	–	Tabet et al. (2004)
<i>Ps. japonica</i>	Leaf	11.3	3.3	<0.1	26.1	14.5	260.0	24.8	Hunter et al. (2003)



**Fig. 5** Relationship between concentrations of trace minerals (a) Mn, (b) Zn, (c) Fe and (d) Cu and shoot height from seven species of bamboo: *Phyllostachys aurea* (PLAR), *Phyllostachys aureasulcata* (PLAU), *Phyllostachys bissettii* (PLBS), *Phyllostachys glauca* (PLGL), *Phyllostachys nuda* (PLNU), *Phyllostachys rubromarginata* (PLRU), *Pseudosasa japonica* (PSJP) collected in 2009. All trace minerals differed ( $p < 0.05$ ) by shoot size and species except for Cu.

Mn and Zn, regulate many processes during plant shoot development (Raven *et al.*, 1992). For example, Fe has been described to be involved in enzyme functions required for new plant cell growth and photosynthesis during early shoot development in other plant species. The specific activities of minerals in bamboo shoots, however, have yet to be described. The reduction in mineral concentrations with shoot elongation may also relate to the dilution of minerals with increased cell mass, and mobilization of minerals from rhizomes through the elongating culm and to the leaves. Because of the relatively lower fibre content, newly emerging bamboo shoots are likely more digestible and minerals more bioavailable than those found in full-height culm and leaves. The bioavailability of minerals to consumers of bamboo shoots, leaf and culm are largely unknown. However, Zn, Cu and Fe have been reported to associate with fibrous components of the cell wall and be inaccessible to consumers in other forage studies (Van Cutsem and Gillet, 1983; Spears, 1994). The bioavailability of Zn is of particular interest as it is required by several enzymes integral to the biochemistry of carbohydrate, nucleic acid and protein metabolism during carnivore digestion (Spears, 1994). Supplementation of Zn, Cu and Mn has been shown to improve canine reproductive performance (Kuhlman and Rompala, 1998) and may also play an important role in improving reproductive performance of giant pandas in the wild and in captivity.

Potassium was the most abundant of all minerals measured, and shoot K concentrations were also greater than those previously reported in bamboo leaves and elongated culms (Table 2). The high K concentration in shoots likely creates the osmotic pressures necessary to draw water into plant cells in promotion of cell enlargement during the spring growing season. Potassium may also be involved in other cell functions including the transfer of sugars and carbohydrates. Young spring grasses and legumes also were reported to contain large amounts of K in the spring compared to other mineral concentrations (Jacobson *et al.*, 1972; Spears, 1994). In contrast to K, shoots had lower Ca content than bamboo culm and leaf (Table 2). Plant cell walls contain Ca to maintain cellular membrane permeability, and as such, Ca ions are highly correlated with lignin (Raven *et al.*, 1992; Torre *et al.*, 1992). Therefore, the overall lower concentrations of Ca in shoots compared to leaf and culm is consistent with the thinner cell walls and incomplete lignin development during early shoot growth. Although K supports mammalian growth and development, an excess of K can cause reduced absorption and possibly deficiencies of other minerals by the con-

sumer (Spears, 1994). Similarly, bonds between Ca and lignin may decrease Ca bioavailability as ingestion of foodstuffs with large proportions of dietary fibre are known to cause decreased absorption of Ca and other nutrients (Davies, 1979; Fernandez and Phillips, 1982; Eastwood, 1992). The relationship of K and Ca to other nutritional components and its impact on mineral bioavailability for bamboo consumers are of particular interest and should be examined further.

When compared with nutrient values in bamboo leaves and lignified culm, it is evident that not only do shoots have greater quantities of fat, crude protein and some minerals, but also that shoot growth is the period of greatest nutrient change within the lifespan of the bamboo plant (Tables 1 and 2). This information improves knowledge of giant panda nutritional ecology and emphasizes the potential nutritional incentives for bamboo plant part selection. A greater understanding of bamboo nutrient content is valuable to captive giant panda management, and our data suggest that bamboo shoots should be included as a major portion of giant panda diets when available. Giant pandas in the wild have a seemingly unlimited food source within the bamboo forests and are able to meet their dietary requirements by selectively foraging specific plant parts and consuming large quantities of bamboo daily (Schaller *et al.*, 1985). Although a significant amount of literature has been accumulated on giant panda biology due to studies on captive individuals, it is difficult to replicate the dietary conditions experienced by the wild population. Reported diets provided to giant pandas in five Chinese zoos during the 1990s consisted of 17–82% bamboo with additional fruit and vegetables, grain products, occasional meat products, and vitamin and mineral supplements formulated to meet the nutritional requirements of canines (Dierenfeld *et al.*, 1995). In contrast, giant pandas in four U.S. zoos consume diets of 87–96% bamboo (Wiedower *et al.*, 2012). Fourteen per cent of the captive giant pandas surveyed in China were diagnosed with stunted development syndrome (SDS), a condition characterized by small body size, dental abnormalities, chronic gastrointestinal (GIT) disease, failure to reproduce, increased occurrence of mucous stools and poor body condition (Janssen *et al.*, 2006). This syndrome is suspected to result from suboptimal nutrition during postnatal development and has accounted for 28% of captive giant panda deaths (Qui and Mainka, 1993; Dierenfeld *et al.*, 1995). A greater inclusion of bamboo in the diets of captive giant pandas will likely improve overall animal health. The addition of bamboo shoots, in particular, may enrich giant panda dietary management as shoots are likely

to be more digestible due to relatively lower fibre content than full-height culm and are also higher in protein and fat. This is especially applicable during periods of growth when SDS and GIT complications are more likely to occur.

Although some light has been shed on the nutritive value of bamboo shoots, many areas of uncertainty remain for further exploration. Bamboo leaves are known to accumulate silica, an inorganic structural constituent in plant cells, although it is hypothesized to cause the leaves to be less palatable to foragers at times of high concentration (Schaller *et al.*, 1985; Lux *et al.*, 2003). How silica concentrations in shoots compare to leaves has not been investigated, and may be relevant to understanding foraging choices of animals that feed on bamboo. Similarly, cyanogenic glycosides are known to exist in high quantities in many bamboo species (Chang and Hwang, 1990; Nahrstedt, 1993) and may impact forage selection in giant pandas. The concentration of cyanogenic compounds in U.S. cultivated bamboo species and plant parts should be explored in future nutrition studies and compared to the concentration of cyanogenic compounds in bamboo species native to China. It also important to take into account that plant mineral content is highly dependent on minerals available in soil. Future bamboo shoot mineral analyses should be adapted to include soil testing at the times of shoot growth and appreciation of mineral variations in the soils of different stand locations. In addition, elevated temperatures are known to negatively influence the nutrient value of forages (Buxton and Fales, 1994). The magnitude and variability of changing temperatures and rainfall of bamboos available for both captive and free-ranging giant pandas are of particular interest in light of anticipated climate changes. Regarding reproductive and nutritional health of bamboo foragers, it would be worthwhile to investigate shoot concentrations of micronutrients that influence reproduction such as Se, P and vitamins A, C, D and E (Allen and Ullrey, 2004). This information is critical to diet formulation for the captive giant panda to promote successful fertility, conception and parturition as shooting season coin-

cides with the single yearly period of ovulation in most female giant pandas. Bamboo shoots may contain unique and valuable properties to support and maintain pregnancy in giant pandas as has been described for other bamboo consumers (Sanders *et al.*, 1981). A factor that limits inclusion of bamboo shoots in captive panda diets during shooting season is lack of availability. To preserve bamboo stand health, new shoots must grow and replace harvested culms. A prospective remedy to this issue is to consider fertilization of bamboo plots used as forage resources. Fertilization has been shown to increase nutritive value, and overall shoot production in certain species of bamboo (non-fertilized:  $3.6 \pm 0.7$  new shoots per plot =  $9 \times 9 \text{ m}^2$ ; fertilized:  $11.9 \pm 1.1$  new shoots per plot; Li *et al.*, 1998). As demonstrated by this and previous studies, bamboo shoots are highly nutritious and biochemically distinct from full-height culm and leaves. Additional measures should be taken to understand the potential connection between shoot nutritive value and giant panda nutritional health, reproduction and development in wild and captive populations.

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### References

- Allen, M. E.; Ullrey, D. E., 2004: Relationships among nutrition and reproduction and relevance for wild animals. *Zoo Biology* **23**, 475–487.
- AOAC, 1995: *Official Methods of Analysis*. Association of Official Analytical Chemists, Washington, DC.
- Budde, E. F., 1952: The determination of fat in baked biscuit type dog foods. *Journal of the Association of Official Analytical Chemists* **35**, 799–805.
- Buxton, D. R.; Fales, S. L., 1994: Plant environment and quality. In: G. C. Fahey (ed.), *Forage Quality, Evaluation, and Utilization*. American Society of Agronomy Inc., Madison, WI, pp. 155–159.
- Chang, J. Y.; Hwang, L. S., 1990: Analysis of taxiphyllin in bamboo shoots and its changes during processing. *Food Science (China)* **17**, 315–327.
- Chang, W.-J.; Chang, M.-J.; Chang, S.-T.; Yeh, T.-F., 2013: Chemical composition and immunohistological variations of a growing bamboo shoot. *Journal of Wood Chemistry and Technology* **33**, 144–155.

- Cherney, D. J. R.; Cherney, J. H.; Lucey, R. F., 1993: In vitro digestion kinetics and quality of perennial grasses as influenced by forage maturity. *Journal of Dairy Science* **76**, 790–797.
- Darlington, J.; Hershberger, T., 1968: Effect of forage maturity on digestibility, intake and nutritive value of alfalfa, timothy and orchardgrass by equine. *Journal of Animal Science* **27**, 1572–1576.
- Davies, N. T., 1979: Anti-nutrient factors affecting mineral utilization. *Proceedings of the Nutrition Society* **38**, 121–128.
- Dierenfeld, E. S., 1997: *Chemical Composition of Bamboo in Relation to Giant Panda Nutrition Linnean Society Symposium Series*. Academic Press, London, UK.
- Dierenfeld, E. S.; Hintz, H. F.; Robertson, J. B.; Van Soest, P. J.; Oftedal, O. T., 1982: Utilization of bamboo by the giant panda. *The Journal of Nutrition* **112**, 636–641.
- Dierenfeld, E. S.; Qui, X.; Mainka, S.; Liu, W. X., 1995: Giant panda diets fed in five Chinese facilities: an assessment. *Zoo Biology* **14**, 211–222.
- Eastwood, M. A., 1992: The physiological effect of dietary fiber: an update. *Annual Review of Nutrition* **12**, 19–35.
- Fernandez, R.; Phillips, S. F., 1982: Components of fiber impair iron absorption in the dog. *American Journal of Clinical Nutrition* **35**, 107–112.
- Halvorson, J. J.; Cassida, K. A.; Turner, K. E.; Belesky, D. P., 2010: Nutritive value of bamboo as browse for livestock. *Renewable Agriculture and Food Systems* **26**, 161–170.
- Hansen, R. L.; Carr, M. M.; Apanavicius, C. J.; Jiang, P.; Bissell, H. A.; Gocinski, B. L.; Maury, F.; Himmelreich, M.; Beard, S.; Ouellette, J. R.; Kouba, A. J., 2010: Seasonal shifts in giant panda feeding behavior: relationships to bamboo plant part consumption. *Zoo Biology* **29**, 470–483.
- Hirayama, K.; Kawamura, S.; Mitsuoka, T.; Tashiro, K., 1989: The faecal flora of the giant panda (*Ailuropoda melanoleuca*). *Journal of Applied Microbiology* **67**, 411–415.
- Hoffman, P. C.; Sievert, S. J.; Shaver, R. D.; Welch, D. A.; Combs, D. K., 1993: In situ dry matter, protein, and fiber degradation of perennial forages. *Journal of Dairy Science* **76**, 2632–2643.
- Hunter, I. R.; Dierenfeld, E. S.; Jinhe, F., 2003: The possible nutritional consequences for giant panda of establishing reserve corridors with various bamboo species. *Journal of Bamboo and Rattan* **2**, 167–178.
- Jacobson, D. R.; Hemken, R. W.; Button, F. S.; Hatton, R. H., 1972: Mineral nutrition: calcium, phosphorus, magnesium, and potassium interrelationships. *Journal of Dairy Science* **55**, 935–944.
- Janssen, D.; Edwards, M.; Sutherland-Smith, M.; Yu, J.; Li, D.; Zhang, G.; Wei, R.; Zhang, C.; Miller, R.; Phillips, L.; Hu, D.; Tang, C., 2006: Significant medical issues and biological reference values for giant pandas from the Biomedical Survey. In: D. E. Wildt, A. Zhang, H. Zhang, D. L. Janssen, S. Ellis (eds), *Giant Pandas: Biology, Veterinary Medicine and Management*, Cambridge University Press, New York, NY, pp. 586.
- Kuhlman, G.; Rompala, R. E., 1998: The influence of dietary sources of zinc, copper, and manganese on canine reproductive performance and hair mineral content. *The Journal of Nutrition* **128**, 2603S–2605S.
- Li, R.; Werger, M. J. A.; During, H. J.; Zhong, Z. C., 1998: Carbon and nutrient dynamics in relation to growth rhythm in the giant bamboo *Phyllostachys pubescens*. *Plant and Soil* **201**, 113–123.
- Liese, W., 1992: The structure of bamboo in relation to its properties and utilization. International Symposium on Industrial Use of Bamboo. International Tropical Timber Organization, Beijing, China.
- Long, Y.; Lu, Z.; Wang, D.; Zhu, X.; Wang, H.; Zhang, Y.; Pan, W., 2004: Nutritional strategy of giant pandas in the Qinling mountains of China. In: D. Lindburg, K. Baragona (eds), *Giant Pandas Biology and Conservation*. University of California Press, Los Angeles, CA, pp. 90–100.
- Lux, A.; Luxova, M.; Abe, J.; Morita, S.; Inanaga, S., 2003: Silicification of bamboo (*Phyllostachys heterocycla* Mitf.) root and leaf. *Plant and Soil* **255**, 85–91.
- Mainka, S. A.; Guanlu, Z.; Mao, L., 1989: Utilization of a bamboo, sugar cane, and gruel diet by two juvenile giant pandas (*Ailuropoda melanoleuca*). *Journal of Zoo and Wildlife Medicine* **20**, 39–44.
- Makita, A., 1998: The significance of the mode of clonal growth in the life history of bamboos. *Plant Species Biology* **13**, 85–92.
- Nahrstedt, A., 1993: Cyanogenesis and foodplants. Chapter 7 In: *Proceedings of the Phytochemical Society of Europe vol. 34*. Phytochemistry and Agriculture, pp. 107–129.
- Nelson, C. J.; Moser, L. E., 1994: Plant factors affecting forage quality. In: G. C. Fahey (ed.) *Forage Quality, Evaluation, and Utilization*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, pp. 115–154.
- Nelson, S. L.; Miller, M. A.; Heske, E. J.; Fahey, G. C., 2000: Nutritional quality of leaves and unripe fruit consumed as famine foods by the flying foxes of Samoa. *Pacific Science* **54**, 301–311.
- Nirmala, C.; David, E.; Sharma, M. L., 2007: Changes in nutrient components during ageing of emerging juvenile bamboo shoots. *International Journal of Food Sciences and Nutrition* **8**, 612–618.
- Pandey, A. K.; Ojha, V., 2013: Standardization of harvesting age of bamboo shoots with respect to nutritional and anti-nutritional components. *Journal of Forestry Research* **24**, 83–90.
- Proscky, L.; Asp, N.-G.; Furda, I.; DeVries, J. W.; Schweizer, T. F.; Harland, B. F., 1985: Determination of total dietary fiber in foods and food products: collaborative study. *Journal of the Association of Official Analytical Chemists* **68**, 677–679.
- Proscky, L.; Asp, N.-G.; Schweizer, T. F.; DeVries, J. W.; Furda, I., 1992: Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. *Journal of the Association of Official Analytical Chemists* **75**, 360–367.
- Qui, X.; Mainka, S. A., 1993: Review of mortality of the giant panda. *Journal of Zoo and Wildlife Medicine* **24**, 425–429.
- Quinn, G. P.; Keough, M. J., 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, New York, NY. 535 pp.
- Raven, P. H.; Evert, R. F.; Eichhorn, S. E. (eds), 1992: *Biology of Plants*. Worth Publishers, New York, NY, 791 pp.
- Sanders, E. H.; Gardner, P. D.; Berger, P. J.; Negus, N. C., 1981: 6-methoxybenzoxazolinone: a plant derivative that stimulates reproduction in *Microtus montanus*. *Science* **4516**, 67–69.
- Schaller, G. J. H.; Wenshi, P.; Jing, Z., 1985: *The Giant Pandas of Wolong*. University of Chicago Press, Chicago, IL.
- Spears, J. W., 1994: Minerals in forages. In: G. C. Fahey (ed.) *Forage Quality, Evaluation, and Utilization*. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, pp. 281–317.

- Suzuki, K.; Itoh, T., 2001: The changes in cell wall architecture during lignification of bamboo, *Phyllostachys aurea* Carr. *Trees* **15**, 137–147.
- Tabet, R. B.; Oftedal, O. T.; Allen, M. E., 2004: Seasonal differences in composition of bamboo fed to giant pandas (*Ailuropoda melanoleuca*) at the National Zoo. *Proceedings of the Fifth Comparative Nutrition Society Symposium*, pp. 176–180.
- Tarou, L. R.; Williams, J.; Powell, D. M.; Tabet, R.; Allen, M., 2005: Behavioral preferences for bamboo in a pair of captive giant pandas (*Ailuropoda melanoleuca*). *Zoo Biology* **24**, 177–183.
- Torre, M.; Rodriguez, A. R.; Saura-Calixto, F., 1992: Study of the interactions of calcium ions with lignin, cellulose, and pectin. *Journal of Agricultural and Food Chemistry* **40**, 1762–1766.
- Triplett, J. K.; Clark, L. G., 2010: Phylogeny of the temperate bamboos (Poaceae: Bambusoideae: Bambuseae) with an emphasis on *Arundinaria* and Allies. *Systematic Botany* **35**, 102–120.
- Van Cutsem, P.; Gillet, C., 1983: Proton-metal cation exchange in the cell wall of *Nitella flexilis*. *Plant Physiology* **73**, 865–867.
- Van Soest, P. J., 1963: Use of detergents in the analysis of fibrous feeds. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Analytical Chemists* **46**, 829–835.
- Van Soest, P., 1975: Physico-chemical aspects of fibre digestion. In: I. Mcdonald, A. Warner (eds), *Digestion and Metabolism in the Ruminant*. University of New England, Sydney, pp. 351–365.
- Van Soest, P. J., 1977: Plant fiber and its role in herbivore nutrition. *The Cornell Veterinarian* **67**, 307–326.
- Van Soest, P.; Wine, R., 1967: Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Official Analytical Chemists* **50**, 50–55.
- Wei, F.; Wang, Z.; Feng, Z.; Li, M.; Zhou, A., 2000: Seasonal energy utilization in bamboo by the red panda (*Ailurus fulgens*). *Zoo Biology* **19**, 27–33.
- Wiedower, E. E.; Kouba, A. J.; Vance, C. K.; Hansen, R. L.; Stuth, J. W.; Tolleson, D. R., 2012: Fecal near infrared spectroscopy to discriminate physiological status in giant pandas. *PLoS One* **7**, 1–10.
- Williams, C. L.; Willard, S.; Kouba, A.; Sparks, D.; Holmes, W.; Falcone, J.; Williams, C. H.; Brown, A., 2013: Dietary shifts affect the gastrointestinal microflora of the giant panda (*Ailuropoda melanoleuca*). *Journal of Animal Physiology and Animal Nutrition* **97**, 577–585.
- Zhu, L.; Wu, Q.; Dai, J.; Zhang, S.; Wei, F., 2011: Evidence of cellulose metabolism by the giant panda gut microbiome. *Proceedings of the National Academy of Sciences* **108**, 17714–17719.